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An epistatic interaction controls the latency of a transgene-induced mammary tumor

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Abstract. Previous studies from our laboratory demonstrated that the latency, tumor growth, and metastatic progression of polyoma middle T-induced mammary tumor in an FVB/NJ inbred mouse background could be significantly altered by the introduction of different genetic backgrounds. In this study we extend these findings by mapping a number of interacting quantitative trait loci responsible for the changes in phenotype. Introduction of the I/LnJ inbred genetic background into the FVB/NJ-PyMT animal significantly accelerated the appearance of the primary tumor (35 vs. 57 days postnatal, $p < 10^{-7}$). A backcross mapping panel was established, and loci responsible for the tumor acceleration were detected on Chrs 15 and 9. Examination of the genotype/phenotype correlation revealed that the FVB/NJ but not the I/LnJ allele of the Chr 15 locus was associated with tumor acceleration and was conditional on the presence of I/LnJ allele on Chr 9. These loci, designated *Apmt1* and *Apmt2*, map to homologous regions associated with LOH in human breast cancer. These results suggest that allelic variants of genes in these regions may contribute to age of onset in human breast cancer.

Introduction

The inherited component of breast cancer was originally observed more than 100 years ago [1] and has been confirmed by the identification of two major susceptibility genes, BRCA1 (Miki et al. 1994) and BRCA2 (Wooster et al. 1995). Women with germline mutations in these genes have a greatly enhanced risk of breast cancer. The likelihood of women carrying mutations in BRCA1 developing breast cancer ranges from approximately 50–80% by the age of 70 (Easton et al. 1993; Struewing et al. 1997), and carries are also at increased risk for ovarian cancer (Narod et al. 1991). Mutations in BRCA2 appear to confer equal risk of breast cancer (Easton et al. 1997) but do not confer as significant an elevated risk of ovarian cancer (Struewing et al. 1997; Ford et al. 1998). Approximately >80% of breast cancer families can be accounted for by the high susceptibility genes (Ford et al. 1998). The high susceptibility genes account for between 10 and 15% of breast cancer in the general population (Newman et al. 1988; Szabo and King 1997).

Although the discovery of these high susceptibility genes is important for understanding the genetic basis of breast cancer, many important questions remain to be answered. The clinical expression of BRCA1 has been shown to vary both between families and within families (Goldgar et al. 1994; Friedman et al. 1995; Langston et al., 1996; Easton et al. 1997). Some women may develop breast cancer early in life, whereas family members bearing the same mutation may remain unaffected until their seventies (Narod et al. 1995). The variable penetrance and age-at-onset observed among individuals bearing the same mutations suggest that there must be additional factors that influence the development of the disease. For example, over-expression of the estrogen receptor has been suggested to be a risk factor (Khan et al. 1998). An allelic variant of the CYP19 gene (aromatase p450) has been found significantly more frequently in breast cancer patients than in control patients and has led to the suggestion that CYP19 is a susceptibility gene with low penetrance (Kristensen et al. 1998). Rare alleles of the HRAS1 gene have also been shown to modify the risk of breast cancer (Krontiris et al. 1993; Phelan et al. 1996). The genes responsible for hereditary non-polyposis colorectal cancer (HPNCC), the candidate tumor suppressor gene of Cowden disease (Liaw et al. 1997), and the ataxia-telangiectasia gene (Athma et al. 1996) have also been associated with breast cancer. Allelic variation in these putative susceptibility genes may, therefore, contribute to the genetic complexity of this disease.

Identifying and characterizing genes that modify breast cancer risk would provide important new insights into the etiology of breast cancer (Ford et al. 1995). At present, however, little is known about breast cancer modifier genes and how they interact with the major susceptibility genes. Owing to the genetic heterogeneity of the human population and interactions with uncontrolled environmental influences, identification of modifiers in human populations can be a difficult task. Because of these difficulties, the mouse has often been used as a model for many human disorders that have both simple and complex genetic components. The mouse has been used successfully for the genetic analysis of many different phenotypes including epilepsy, diabetes, obesity, pigmentation, lupus, alcohol or drug preference, as well as cancer (reviewed in Frankel 1995). The availability of large numbers of inbred strains, high-resolution mapping reagents, and the ability to dissect complex qualitative and quantitative traits make the mouse a valuable tool for analysis of the complex genetic basis of mammary carcinogenesis.

Previously we reported the identification of a number of mouse inbred strains in which the initiation and progression of a transgene-induced mammary tumor was significantly different from the typical pattern (Lifsted et al. 1998). FVB/N-TgN(MMTVPyVT)^{634Mul} mice bear a Polyoma Middle T antigen gene that is expressed under the control of a mouse mammary tumor virus enhancer and promoter (Guy et al. 1992). Female animals inheriting this transgene develop palpable, synchronous, multifocal mammary tumors in all of the mammary glands at approximately 60 days of age (Guy et al. 1992). F_1 progeny of a cross between the FVB/N-TgN(MMTVPyVT)^{634Mul} and the inbred strain I/LnJ, however, have an average latency of only 36 days of age. These data strongly suggest the presence of mammary tumor susceptibility alleles in the I/LnJ strain. We therefore, initiated both genetic

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and biochemical studies to assess the basis for the tumor acceleration in the I/LnJ F_2 animals. We demonstrate that the change in tumor latency cannot be explained by alterations in expression of the transgene, and thus the alteration in tumor latency is most likely due to an increased sensitivity of the I/LnJ mammary gland to polyoma middle T-induced tumorigenicity. Quantitative trait locus (QTL) analysis of an I/LnJ backcross identified at least two interacting genetic regions associated with the increased susceptibility to mammary tumors. None of the genes previously associated with breast cancer susceptibility map to these chromosomal intervals. The I/LnJ alleles may, therefore, represent novel breast cancer susceptibility genes.

Materials and methods

Animals. FVB/N-TgN(MMTVPyVT)^{634Mul} mice were obtained from W. Muller, (McMaster University, Hamilton, Ontario, Canada). FVB/NJ and I/LnJ mice were purchased from The Jackson Laboratory. The backcross (N_2) animals were generated by breeding FVB/N-TgN(MMTVPyVT)^{634Mul} males to I/LnJ females and crossing the transgene-positive males F_1 back to FVB/NJ females. Inheritance of the polyoma transgene was determined by PCR amplification of weanling tail biopsy DNA. Diagnosis of mammary tumors was performed by palpation (~3 mm in diameter). Animals were checked for tumors every other day. After the initial identification of the primary tumor, animals were further aged to confirm the diagnosis.

Antisera. Monoclonal anti-Polyoma T Antigen antibodies were obtained from Oncogene Research Products (San Diego, CA). Polyclonal rat anti-polyoma T antigen antibodies were kindly provided by T. Benjamin (Harvard University, Cambridge, Mass.). HRP-conjugated anti-rat antibody was obtained from Santa Cruz Biotechnology.

Western blot analysis. Tumors were homogenized in lysis buffer (1 × PBS, 1% Triton ×100, 0.5% deoxycholate, 0.1% SDS, 0.004% NaF, 100 μg/ml PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 2mM NaOrthovanadate, pH 7.4) and centrifuged at 100,000 *g* to pellet insoluble material. The protein concentration of the supernatant was determined by the BCA method (Biorad, Hercules, CA). Western blots were performed basically as described (Ausubel et al. 1997). Briefly, 25 μg of protein of each sample was electrophoresed on a 10% SDS-PAGE gel and electroblotted onto an Optitran membrane (Schleicher & Schuell). The filter was blocked in 5% gelatin in TBST (1.5M NaCl, 0.1 M Tris pH 7.5, 0.1% Tween) for 1 h, washed in TBST, and incubated with a 1:500 dilution of the rat polyclonal antibody in TBST. The filter was washed and incubated with a 1:5000 dilution of the secondary anti-rat HRP-conjugated antibody. The ECL detection system (Amersham) was used for detection, and the filter was exposed on Hyperfilm for 5 min.

Immunohistochemistry. Tumors were isolated from animals 40 days after detection. Tissues were fixed in neutral buffered formalin, paraffin embedded, and sectioned. Immunohistochemical stains were performed with a Vectastain Elite ABC kit (Vector Laboratories, Burlingham, Calif.), following the manufacturer's protocol.

Genotyping. Tail biopsy DNA was used as a template for PCR reactions. Microsatellite primers were purchased from Research Genetics (Huntsville, Ala). PCR reactions were performed basically as described (Dietrich et al. 1992). Reactions were performed in a PTC200 Thermocycler (MJ Research, Watertown, Mass.) and analyzed on 4% agarose TAE gels. The following loci were used: *D1Mit1, D1Mit33, D1Mit46, D1Mit105, D2Mit1, D2Mit113, D2Mit277, D3Mit29, D3Mit147, D3Mit224, D4Mit9, D4Mit17, D4Mit18, D4Mit200, D4Mit214, D4Mit256, D4Mit308, D4Mit348, D5Mit81, D5Mit201, D5Mit223, D5Mit247, D6Mit14, D6Mit15, D6Mit123, D6Mit138, D7Mit44, D7Mit76, D7Mit76, D7Mit109, D7Mit232, D7Mit246, D8Mit191, D8Mit215, D9Mit82, D9Mit129, D9Mit182, D9Mit207, D9Mit355, D10Mit16, D10Mit95, D10Mit186, D11Mit4, D11Mit214, D11Mit231, D12Mit10, D12Mit134, D12Mit201, D13Mit16, D13Mit171, D13Mit202, D14Mit120, D14Mit170, D15Mit105, D15Mit179, D15Mit184, D15Mit193, D16Mit32, D16Mit139, D17Mit81, D17Mit93, D17Mit139, D17Mit177, D18Mit60, D18Mit142, D19Mit71, D19Mit79, D19Mit88, DXMit140.*

Data analysis. The dataset was mapped by using Map Manager QT (Manly and Olson 1999). Appropriate statistical thresholds ($p = 0.5$, 0.05, and 0.01) for mapping QTLs (Lander and Kruglyak 1995) were estimated by permuting the correctly ordered dataset 10,000 times by using the Doerge and Churchill (1996) algorithm implemented by Map Manager. Each permutation was mapped at 1-cm intervals across the entire genome.

Statistical analysis. The epistatic interaction of the two loci was assessed under the assumption that each locus has a predominantly additive effect on response (latency). An interaction between loci would then mean that the magnitude of the additive effect at one locus depends on the level of the other locus. This hypothesis was formally tested via analysis of variance. The analysis of variance requires the assumption of homoscedasticity (i.e., assume that latency has the same variance for each of the four genotypes). This assumption was supported by the similarity of the four sample standard deviations. Consequently, the results were based on the original data: no variance stabilizing transformation was implemented. The dependent variable was tumor latency, and the additive main effect of each individual locus was modeled through an indicator variable for locus genotype. The main effect of each locus (the additive effect of one locus on latency averaged over the levels of the other locus), as well as the interaction between the two loci, was then estimated and tested. Physically, the interaction term represents the difference in the additive effect of one locus at the two levels of the other locus. Additional statistical analysis was performed with Quick Statistica Package (Statsoft, Tulsa, Okla).

Results

Analysis of transgene expression in mammary tumors. In order to rule out the possibility that the change in tumor latency (Fig. 1) was due to a difference in post-transcriptional control of the transgene, we performed Western blots. Total protein was isolated from tumors and probed with anti-polyoma T antibodies. As can be observed in Fig. 2, multiple bands were observed at the anticipated size of approximately 56 kilodaltons in the polyoma middle T-positive samples (Guy et al. 1992). The two bands presumably represent different phosphorylation states of the protein. No difference in the levels of the polyoma middle T antigen was observed between the FVB/N or [I/LnJ × FVB/NJ]F_1 tumors. The equal intensity ratio of the two polyoma middle T bands present in the samples also suggests that there is no difference in the post-translational modification of the protein between the FVB/N or [I/LnJ × FVB/NJ]F_1 tumors.

Determination of transgene temporal expression. The acceleration of tumor latency might also have been explained by different temporal expression patterns of the transgenes in the different genetic backgrounds. To address this possibility, we performed immunohistochemical analyses. Previous studies from other laboratories demonstrated the presence of pathological abnormalities present in the mammary glands of 21-day-old FVB/N animals, indicative of transgene expression at that age. Therefore, mammary glands from 5- and 10-day-old transgene-positive female [I/LnJ × FVB/NJ]F_1 females were harvested, sectioned, and in situ immunohistochemical stains with the anti-polyoma middle T antibodies performed. No expression of the middle-T antigen was detected in the 5-day-old mammary gland (data not shown). Middle-T protein was detected in the 10-day-old gland (see Fig. 3). Some, but not all of the epithelial ducts stained with the antibody. In addition, some of the ducts showed sectored expression (see Fig. 3). These data suggest that the transgene was being translationally activated in the mammary epithelium of the 10-day-old [I/LnJ × FVB/NJ]F_1 animals. Similar expression patterns of the middle-T antigen was observed in 10 day old transgene-positive FVB/NJ animals, demonstrating that the acceleration of the [I/LnJ × FVB/NJ]F_1 tumors could not be simply explained by the transgene being expressed 20 days earlier.

The extent of the transgene expression in the 10-day-old mammary glands was also assessed in the 10-day-old mammary glands to determine whether there was more extensive expression of the PyMT in the I/LnJ F_1 animals. Serial sections of 10-day-old mammary glands were made from FVB/NJ ($n = 4$) and [I/LnJ × FVB/NJ]F_1 ($n = 6$) animals and stained for PyMT expression. The percentage of PyMT-positive epithelial ducts for each section was determined. Of the FVB/NJ ducts, 26% (28/105) observed were PyMT-positive, compared with 17% of the I/LnJ F_1 ducts (16/94). This result was found not to be significantly different by the Mann Whitney U test ($p = 0.15$). It is, therefore, unlikely that the transgene is upregulated significantly earlier in the I/LnJ F_1 than the FVB/NJ animals,

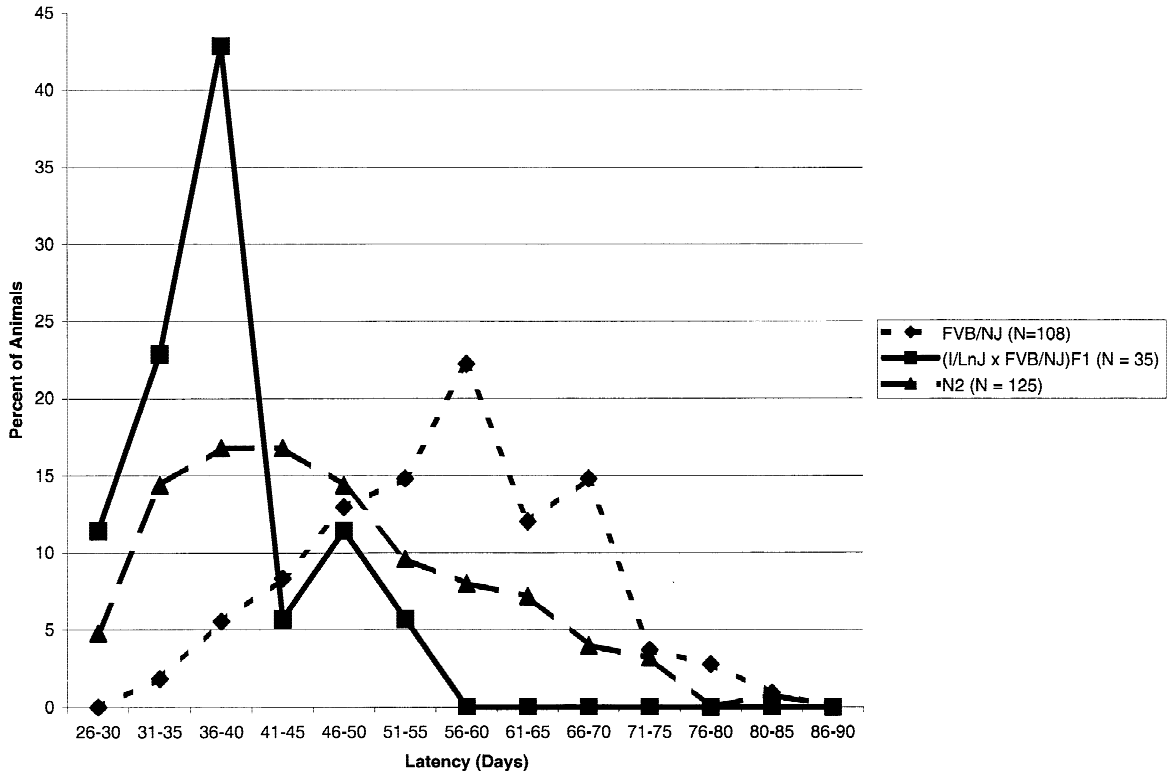

Fig. 1. Latency distribution of FVB/NJ-TgN(MMTV-PyMT)^{634Mul}, I/LnJ F_1, and (I/LnJ × FVB/NJ) × FVB/NJ N_2 animals. The X-axis represents the number of days after birth at diagnosis of the primary mammary tumor. The Y-axis represents the percentage of animals in each class in each latency range.

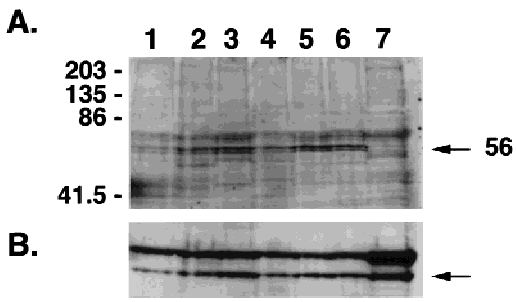

Fig. 2. Comparison of tumor latency with transgene expression in N_2 animals. Total protein extracts prepared from tumors probed with antibody against **A)** Polyoma middle T antigen. The latencies of the animals, in days after birth, are as follows: lanes 1–3: 29 days; lane 4: 74 days; lane 5: 74 days; lane 6: 80 days; lane 7: non-transgenic control. The arrow on the right side indicates the expected 56-kDa size for the polyoma middle T antigen. **B)** α-tubulin. The arrow on the right side indicates the 50-kDa α-tubulin band. The positions of the size markers are indicated on the left side of the figure.

since one would expect more extensive expression. These results do not, however, rule out an effect on latency of a few days' difference in transgene upregulation, although the 3-week difference in latency can not be explained by a 3-week difference in transgene expression.

QTL mapping. Taken together, these data strongly suggested that the acceleration of the mammary tumorigenesis observed in the [I/LnJ × FVB/NJ]F_1 animals was due to the presence of at least one dominant mammary tumor susceptibility allele(s) in the I/LnJ background. A backcross was generated to identify the genomic region or regions that harbored the susceptibility gene(s). Transgene-positive FVB/NJ males were bred to I/LnJ females, and the transgene-positive F_1 males were crossed back to FVB/NJ females. Since the original experiment was designed to detect dominant alleles affecting mammary tumorigenesis (Lifsted et al. 1998), the reciprocal backcross was not performed because of the possibility of recessive alleles in the I/LnJ background that might have interfered with the analysis. 126 female backcross (N_2) animals were generated, the tumor latency was determined, and a genome scan was performed with 69 loci (average spacing ~20 cM). The haplotype and latency data were analyzed with the computer program Map Manager QT to identify loci associated with the acceleration of the tumor latency. One interval that exceeded the suggested statistically significant threshold (LOD = 3.3) was identified near the *D15Mit184* microsatellite locus (Fig. 4). This locus was responsible for approximately 7% of the variance observed. We have designated this locus *A*ccelerator of *P*olyoma-Induced *M*ammary *T*umors (*Apmt1*). A second locus on Chr 7 approached but did not exceed the recommended statistically significant threshold. A third suggestive interval was identified on Chr 9 near the locus *D9Mit182*.

Analysis of epistatic interaction. Examination of the genotype by phenotype correlation of *Apmt1* in the extreme 25% of the latency distribution demonstrated an over-representation of the FVB/NJ allele in the fast arising tumors (latency 36 ≤days; 23/31) and an exclusion of the FVB/NJ allele from the long latency tumors (latency ≥56 days; 10/31; $\chi^2 = 10.9$, 1 d.f., $p = 0.0009$). This was unexpected, since the original strain survey experiment was originally designed to detect dominant loci contributed by the I/LnJ genome (Lifsted et al. 1998). This suggested the possibility that the change in tumor latency was generated in large part by an epistatic interaction of an unknown I/LnJ allele with the FVB/NJ allele of *Apmt1*. Genotype data were reexamined to discover heterozygous loci that were strongly associated with tumor acceleration in FVB/NJ animals that were likely to be *Apmt1* homozygotes. There was a strong correlation between tumor latency and the interval on Chr 9 (see above). Animals that were heterozygous for *D9Mit182* and homozygous for *D15Mit184* had the greatest acceleration of the tumor compared with FVB/NJ parents (38.7 days versus 56.6 days, $p < 10^{-7}$; see Table 1). All four genotype classes were significantly different from the FVB/NJ parent, indicating the presence of at least one additional modifier gene. However, no other combinations that we examined demonstrated such a significant association. Therefore, we have designated the Chr 9 locus *Apmt2*.

The effect of the *Apmt1* and *Apmt2* interaction was determined by comparing the effect of the four genotype classes with each other. As can be seen in Table 2, animals homozygous for both loci were not signifi-

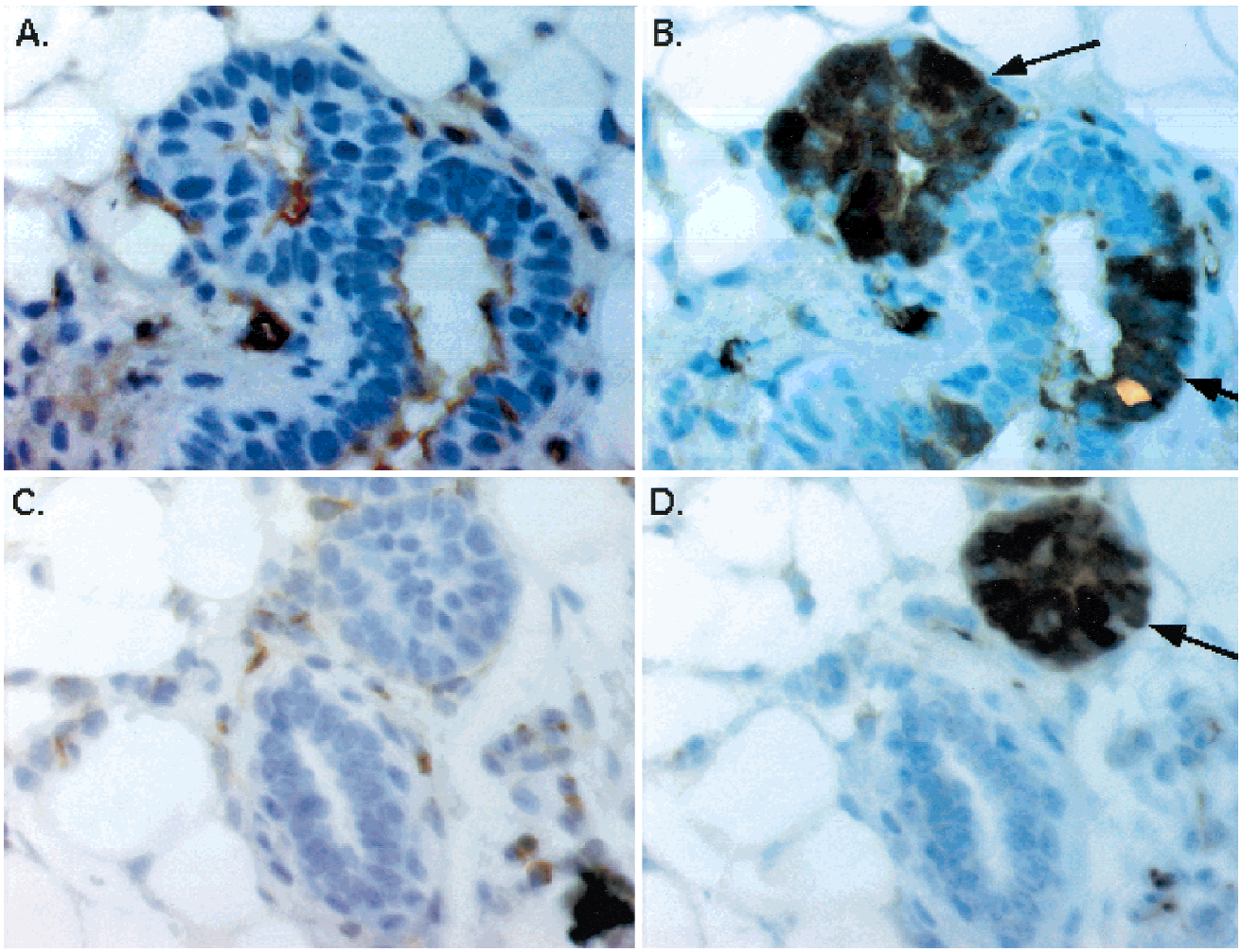

Fig. 3. Example of polyoma middle T immunostain of 10-day-old mammary glands. Adjacent serial sections of paraffin-embedded mammary glands stained with or without an anti-PyMT monoclonal antibody. **A:** I/LnJ F_1 mammary gland, no antibody control; **B:** I/LnJ F_1 mammary gland, with PyMT antibody; **C:** FVB/NJ mammary gland, no antibody control; **D:** FVB/NJ, PyMT antibody. The arrows indicate PyMT staining of the glandular epithelia.

cantly different from animals homozygous for Chr 9 and heterozygous for Chr 15 (row A). Conversely, there was a significant difference between the double heterozygotes and the Chr 9 heterozygous / Chr 15 homozygous animals (row B). These data suggest that *Apmt1* on Chr 15 acts in an additive manner with each FVB/NJ allele accelerating tumor latency approximately 10 days, but is conditional on the presence of an I/LnJ allele at *Apmt2*.

This putative interaction was further explored by analyzing the role of the Chr 9 locus in the Chr 15 homozygous subset of animals. If the Chr 15 locus was dependent upon the presence of an I/LnJ allele on Chr 9, the Chr 9 locus would be expected to be highly significant in that population. The effect of the Chr 9 locus was, therefore, examined in the 62 Chr, 15 homozygous animals. A Chi-squared analysis demonstrated that the association of the I/LnJ allele on Chr 9 with tumor latency was highly significant ($p = 0.00009$) and accounted for 20% of the variance in this population.

The putative epistatic interaction was formally analyzed by analysis of variance. If the effect of each locus on tumor latency was primarily additive and there was no epistatic interaction, then the difference in the Chr 9 FF/Chr 15 FF and Chr 9 FI/Chr 15 FF animals is expected to be the same as that between the Chr 9 FF/Chr 15 FI and Chr 9 FI/Chr 15 FI animals. ANOVA analysis demonstrated that the estimated difference for the Chr 15 FF animals (49.4–38.7 = 10.7 days) was significantly greater than the difference among Chr 15 FI animals (estimated as 50.8–48.6 = 2.2 days; $p = 0.03$), suggesting that these loci interact epistatically. Together, the interacting loci account for approximately 17% of the variance observed.

Discussion

The intended goal of this project was to identify genes that had a dominant effect on the ability of the transgene-induced mammary tumor to form. In previous studies, we had performed a strain survey to identify inbred strains, including I/lnJ, whose genomes would significantly alter the initiation or progression of the tumor in F_1 hybrid animals. We therefore had anticipated that the gene or genes mapped in the current study would be of I/LnJ origin acting in a simple dominant manner. The identification that the FVB/NJ allele *Apmt1* on Chr 15 was acting additively to reduce latency was therefore unexpected. This locus, however, required the presence of at least one I/LnJ allele elsewhere in the genome that was interacting with *Apmt1*. Since we had performed a backcross, it was possible to identify *Apmt2* on Chr 9. An intercross would have required the examination of nine different genotype classes instead of the four produced in the backcross. It is unclear whether we would have been able to easily identify *Apmt2* with the number of animals used.

Two possible models would explain the genetic interaction observed in this study. The first model would be that the Chr 9 locus was dependent on a recessive allele at the Chr 15 locus and exerted its effect only when Chr 15 was homozygous for the FVB/

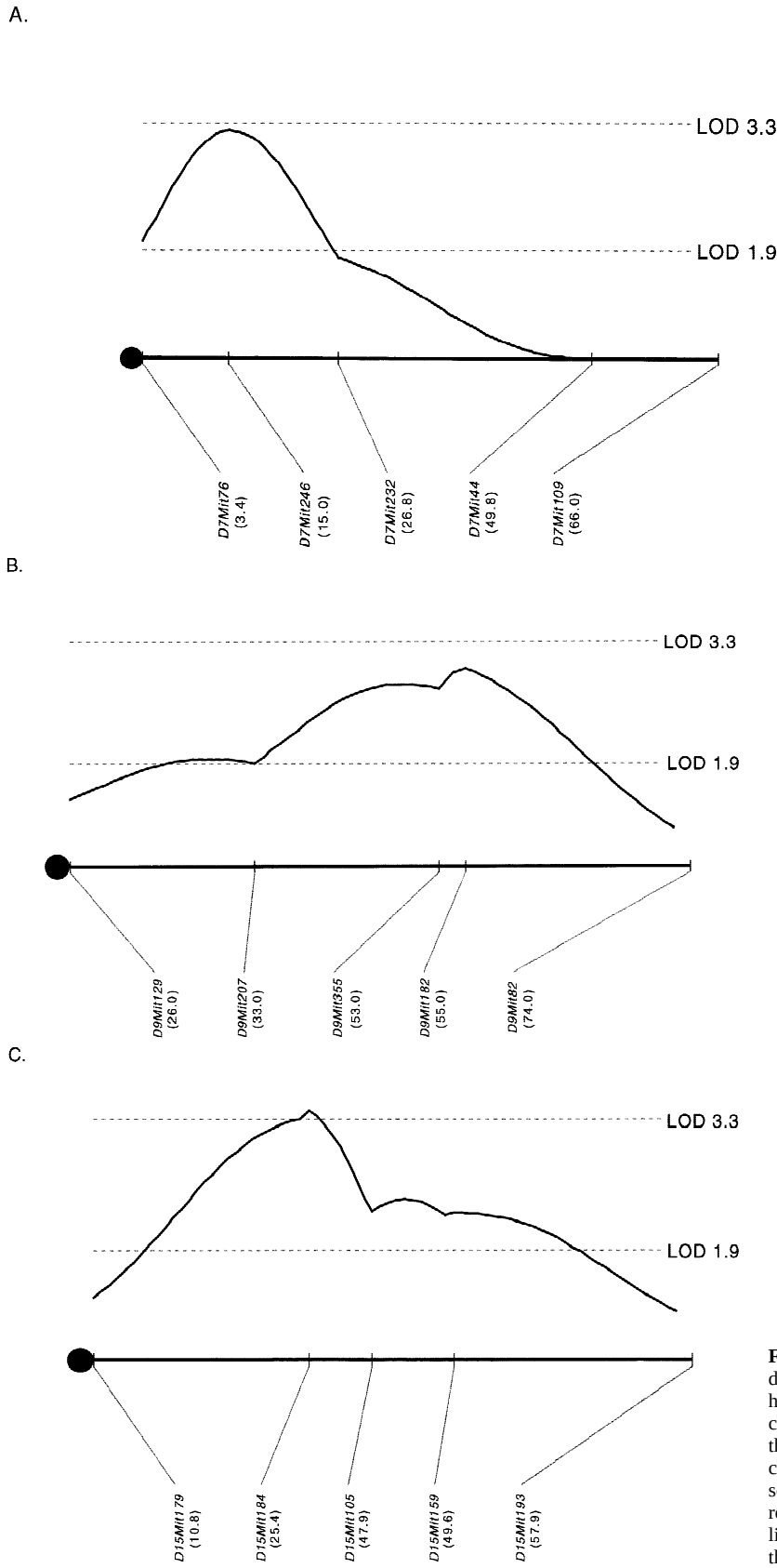

Fig. 4. Results of the genome-wide QTL scan for an additive model. The strategy is described in the text. The heavy horizontal line depicts the chromosomes with the centromeres to the left. The MGD designated position of the loci, in centimorgans, along the chromosome is indicated below the locus designation in parentheses. The loci scored are listed below the chromosome. The dashed lines represent the statistically significant thresholds. The lower line represents the suggestive and the upper the significant threshold. **A:** Chr 7; **B:** Chr 9; **C:** Chr 15.

Table 1. Comparison of FVB/NJ, F_1, and N_2 latencies.

Locus/Genotype	FVB/NJ	I/LnJ F_1	N_2 Genotypes			
D9Mit182 (*Apmt2*)	–	–	FF	FI	FF	FI
D15Mit184 (*Apmt1*)	–	–	FF	FF	FI	FI
Average Latency	56.6	37.3	49.4	38.7	50.8	48.6
S.E.M.	1.0	1.1	2.4	1.3	2.0	2.0
N	108	35	28	34	30	34
P	1	$<10^{-7}$	0.01	$<10^{-7}$	0.04	0.002

P values were determined by comparing the latency data of each genotype class against the FVB/NJ parent with the Mann Whitney U test, corrected for multiple tests.

Table 2. Two-factor analysis of variance results.

Factor	DF	Sum of Squares	Mean Square	F	*P* Value
Main effect Chr 9	1	1187.5	1187.5	10.9	0.001
Main effect Chr 15	1	1250.1	1250.1	8.6	0.004
Interaction	1	573.2	573.2	4.89	0.029
Residual	122	14289.0	117.1		
Total	125	17299.7			

DF = degrees of freedom; F = F-ratio.

NJ allele. The other model would be that the Chr 15 FVB/NJ allele acts additively on latency, but only in the presence of an I/LnJ allele on Chr 9. We favor the latter explanation for the following three reasons. First, the acceleration of latency was observed originally in an F_1 population; therefore, the major modifier loci can not be operating as recessives. Secondly, the *Apmt1*/*Apmt2* compound heterozygous animals, which are most analogous to the F_1 population, are highly significantly different from the FVB/NJ parents ($p = 0.002$; see Table 1), suggesting that these loci play a significant role in the acceleration of the disease. However, since these animals do not have as severe a phenotype as the F_1 population, there are almost certainly other modifiers present, probably including the Chr 7 locus. Finally, if the Chr 15 locus is recessive, then there would have to be other major modifier loci to explain the tumor acceleration observed. Only the three loci described, on Chrs 7, 9, and 15, were observed in our analysis, even at the low stringency genome-wide *p* threshold of 0.05, arguing against the presence of additional major modifier loci. Thus, although there are likely to be other possible explanations, we feel the most likely model would be that the second model proposed, i.e., the FVB/NJ allele of *Apmt1* on Chr 15, interacts additively with the I/LnJ allele of *Apmt2* on Chr 9.

The acceleration of the tumor latency in the I/LnJ animals was of particular interest because of the known sensitivity of I/LnJ mammary glands to hormones (Singh et al. 1970). Explanted I/LnJ mammary glands exposed to exogenous hormones in organ cultures were shown to develop more rapidly than other inbred strains. Endogenous hormone levels are thought to play an important role in the development of breast cancer (Vessey 1997; Helzlsouer and Couzi 1995). In addition, use of exogenous hormones (Hulka 1997; Ursin et al. 1997) and oral contraceptives (Andrieu et al. 1995) have also been implicated in the etiology of breast cancer. We therefore determined whether any of the hormones or hormone receptors important for mammary development mapped to the *Apmt1* or *Apmt2* candidate regions. The estrogen receptor α and β chains are on Chr 10 and 12 respectively (MGD, http://informatics.jax.org). *Cyp19* and *Cyp11a*, the enzymes that catalyze the biosynthesis of estrogen and progesterone, are on Chr 9 but located proximal of the *Apmt2* candidate region. The prolactin gene is on Chr 13. The prolactin receptor maps to the proximal end of Chr 15, but is not included in the *Apmt1* candidate region. The progesterone receptor gene maps to human Chr 11q22, which is homologous with proximal mouse Chr 9. This region, however, is not contained in the *Apmt2* candidate region and therefore excludes the progesterone receptor as a candidate for *Apmt2*. Combined, these data suggest that the acceleration of tumor latency in the I/LnJ animals is not owing to a direct effect of these hormones or receptors, although an indirect effect has not been ruled out.

We have examined a number of other loci commonly associated or suspected to be associated with breast cancer as potential candidates for *Apmt1* and *Apmt2*. *Brca1*, *Brca2*, *Trp53*, and *Erbb2*, genes associated with hereditary breast cancer or genetic abnormalities in sporadic cancers, map to Chr 11. *Brca2* is located on Chr 5. *Hras1* is on Chr 7. These loci are, therefore, not responsible for the increased susceptibility of the I/LnJ mice to polyoma middle T-induced mammary tumors. *Myc*, which is often amplified in breast cancer (Bieche and Lidereau 1995), is located within the *Apmt1* candidate region and therefore might be considered a candidate locus.

Since we have excluded most of the known or suspected breast cancer susceptibility loci, *Apmt1* and *Apmt2* may, therefore, represent novel breast cancer susceptibility genes. However, polyoma virus, the proximal cause of tumors in this model, to the best of our knowledge is not associated with breast cancer. It is possible that the loci detected in this study are specific to the polyoma middle T pathway and have no role in human cancer. While it is not possible at this time to rule out this possibility, it is encouraging to note that the *Apmt1* and *Apmt2* candidate regions are homologous with human chromosomal regions commonly deleted in breast cancer (8p and 3p, respectively; Anbazhagan et al. 1998; Sekido et al. 1998). Ultimately, the identification of these genes will be required before an unambiguous answer about their role in human cancer can be determined. Towards this end we are currently pursuing several different strategies, including the generation of congenic mouse strains to further delineate the *Apmt1* and *Apmt2* candidate regions, to identify these modifiers of breast cancer induction.

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